# Studies to Find the Causal Agent for Confusing Foliar Lesions in Citrus

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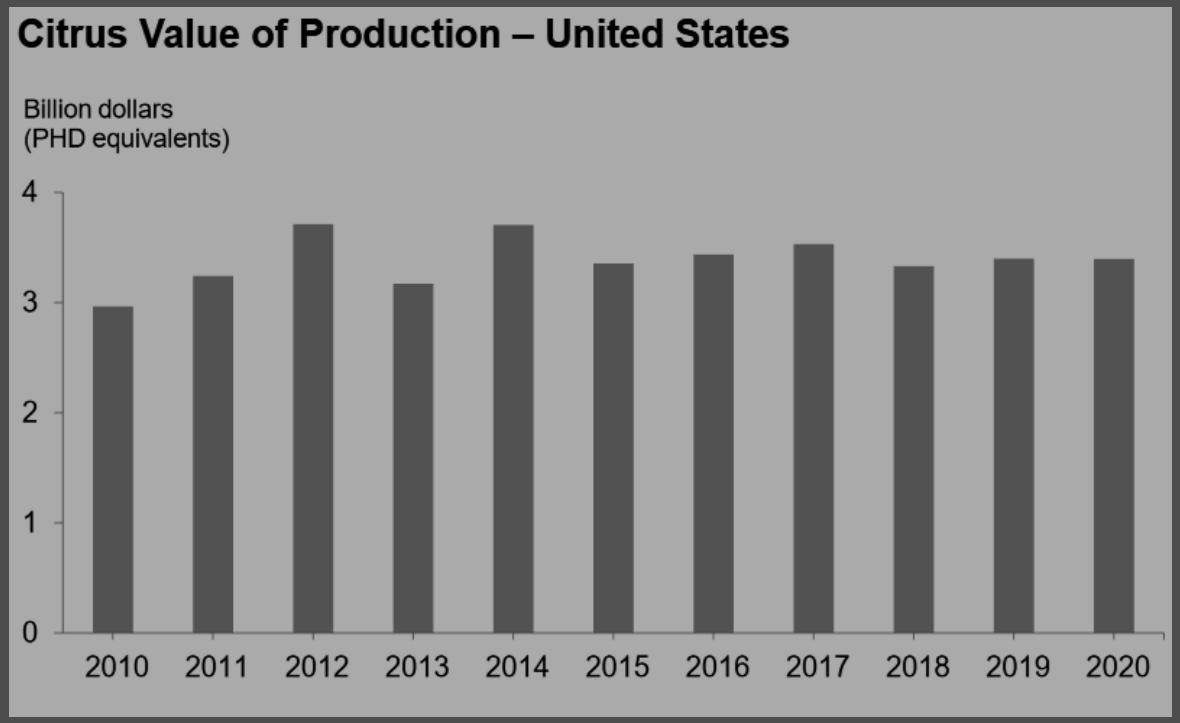
# Citrus Industry (2019)




Citrus Fruits 2020 Summary, United States Department of Agriculture, 2020

- **Worldwide - 143,755.6 thousand tons of citrus produced**
  - **Oranges – 76,292.6 thousand tons**
  - **Tangerines – 37,429.3**
  - **Lemons and limes – 20,529.6**
  - **Grapefruits – 9,504.1**
- **United States - 74.7 thousand tons of citrus produced**
  - **Oranges – 4,832.6 thousand tons**
  - **Tangerines - 986.1**
  - **Lemons and limes - 876.3**
  - **Grapefruits - 511.7**
- **Texas - 233 thousand tons of citrus produced**
  - **Oranges - 1,150,000 boxes**
  - **Grapefruits - 4,400,000 boxes**

# Introduction

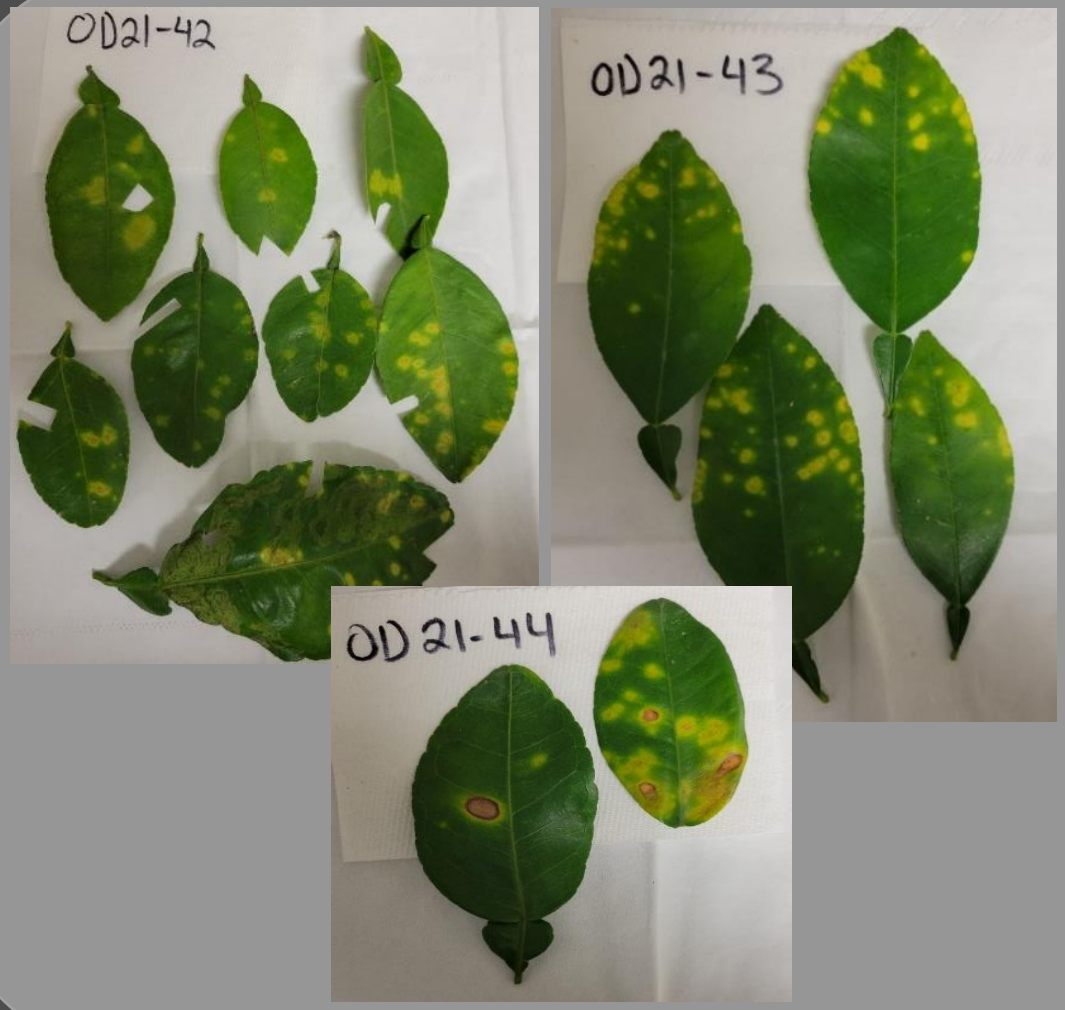
- **In the field, citrus leaves were observed to have lesions that can be confused for symptoms of citrus leprosis virus (CiLV).**
- **Leaves were collected by Dr. Kunta and Ms. Marissa Gonzalez at the citrus center, both from the field and greenhouse**
- **When the leaves were tested by qPCR the samples would be negative for CiLV**

# Citrus Leprosis Virus (CiLV)

- **CiLV is spread by spider mites, genus *Brevipalpus* or flat mites. When a mite (carrier of the virus) feeds on citrus tissue the plant will become infected.**
- **CiLV is mainly found in South and Central America. It has been found as far north as southern Mexico.**
- **CiLV was found in Florida in the 1950's however, has not been seen in the United States since the early 1960s**
- **Sweet orange varieties are more susceptible to the virus**


- United States Department of Agriculture Beltsville Agricultural Research Center Molecular Plant Pathology Laboratory 2016
- Recovery Plant for Citrus Leprosis, United States Department of Agriculture, 2013

# Citrus Leprosis Virus (CiLV)




- United States Department of Agriculture Beltsville Agricultural Research Center Molecular Plant Pathology Laboratory 2016
- Recovery Plant for Citrus Leprosis, United States Department of Agriculture, 2013

# Research Question & Hypothesis

- **What is the cause of the lesions?**
- **Since the results from testing are negative the cause of the lesions is unknown.**
- **It is possible that cause could be a**
  - **fungal infection**
  - **insecticide or herbicide spray damage**
  - **a combination of these two**

# Pesticide

- **Badge SC:** Fungicide & Bactericide
  - **active ingredient:**
    - **Copper Oxychloride – 16.81%**
    - **Copper Hydroxide – 15.36%**
  - **EPA Reg. No. 80289-3-10163**
- **Danitol 2.4 EC:** Insecticide (& miticide)
  - **active ingredient:**
    - **Fenpropathrin – 30.9%**
  - **EPA Reg. No. 59639-35**
- **Minecto Pro:** Insecticide (& miticide)
  - **active ingredient:**
    - **Cyantraniliprole – 12.70%**
    - **Abamectin – 2.68%**
  - **EPA Reg. No. 100-1592**
- **Nealta:** Insecticide (miticide)
  - **active ingredient:**
    - **Cyflumetofen – 18.7%**
  - **EPA Reg. No. 7969-336**

Badge® SC
Bactericide/Fungicide

DANITOL®
2.4 EC SPRAY

Minecto Pro

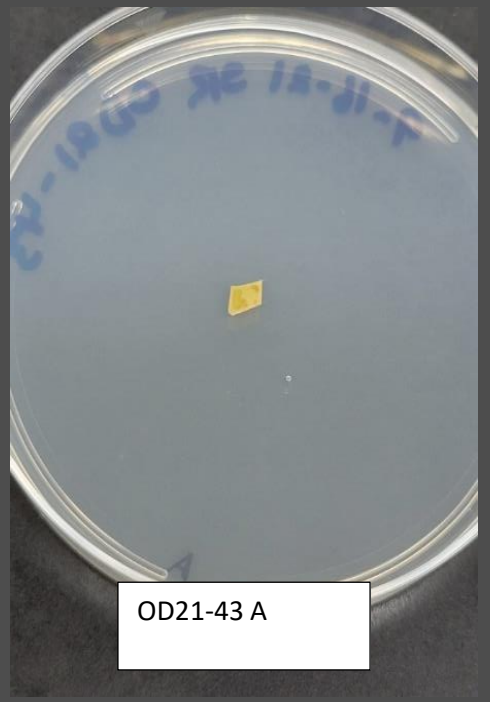
Nealta
Miticide

# Fungal Isolations

- **Lesions were excised from sample leaves and surface disinfected for isolation of fungi on Potato Dextrose Agar (PDA)**
- **From the plated lesions, only those from sample OD21-42 and OD21-44 had growth.**
  - **Samples OD21-42 and OD21-44 both came from the field**
  - **Fungal growth from all plates were observed to be the same.**
- **The lesions from sample OD21-43 had no growth.**
  - **Sample OD21-43 came from a greenhouse.**
- **The lesions is suspected to come from chemical damage due to the pesticides used and the fungus could be a secondary infection.**

OD21-42 A

OD21-42 B



OD21-43 A

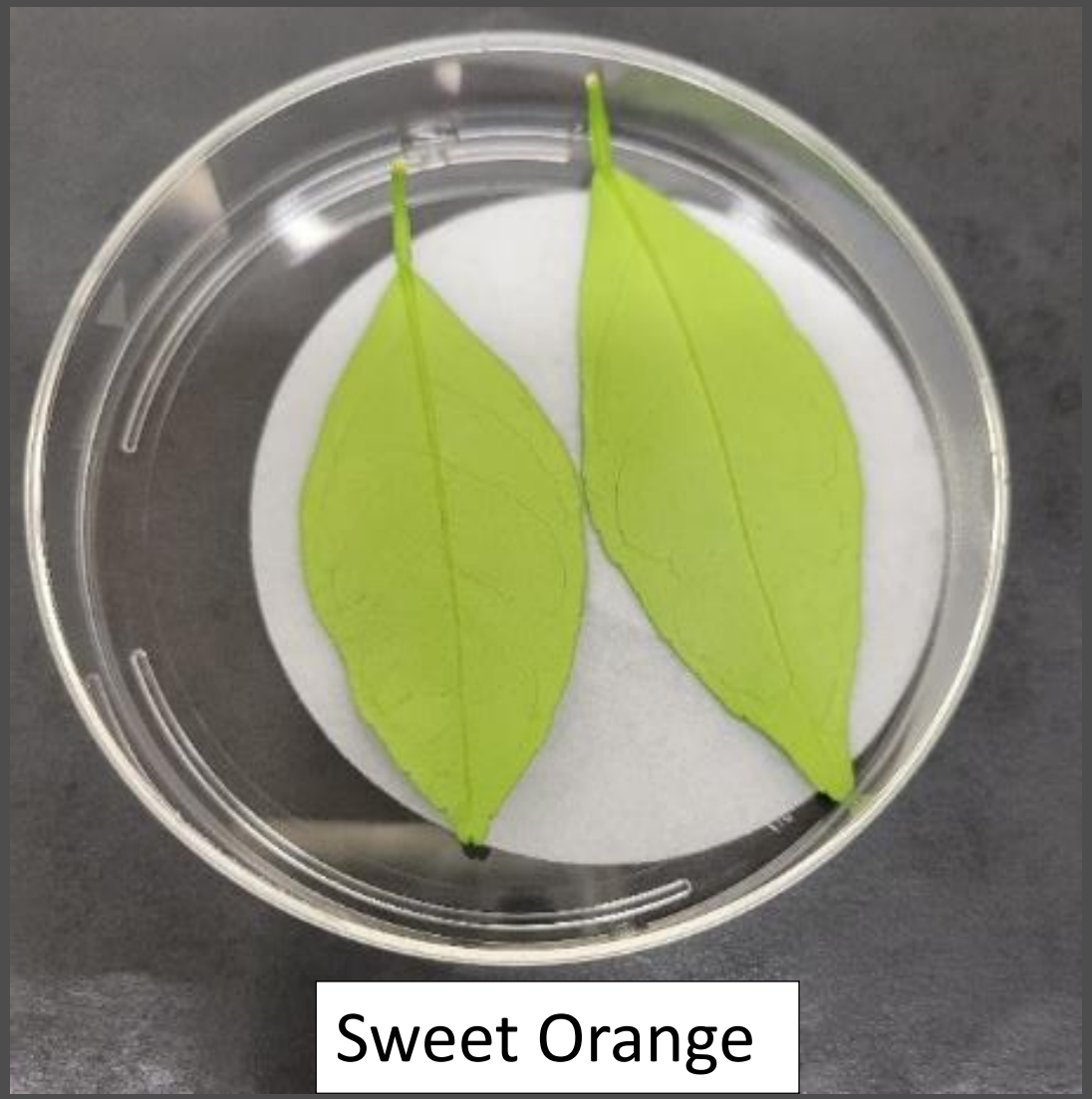
OD21-44 A

OD21-44 B

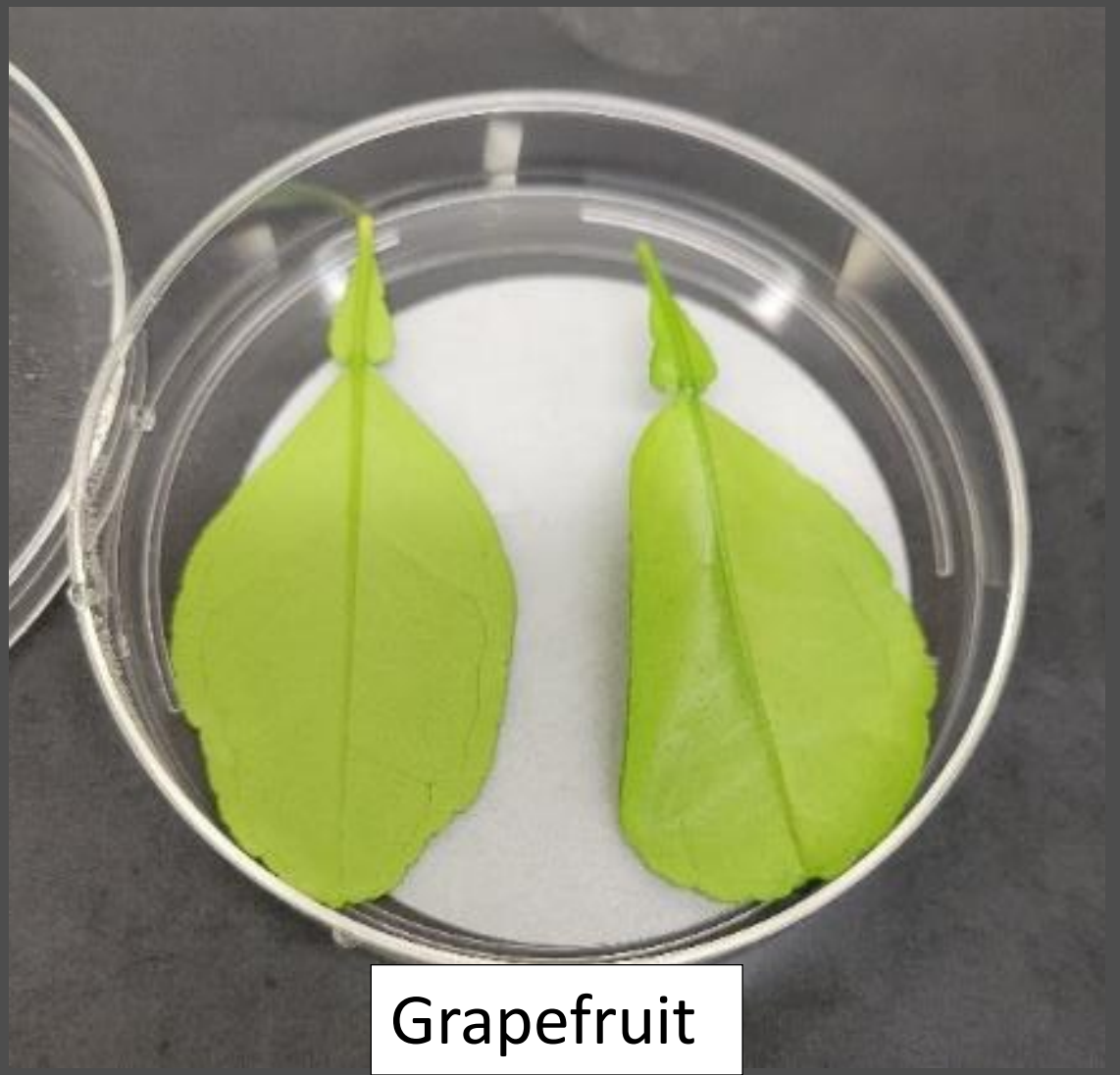
# Leaf Inoculation


- **Healthy grapefruit (GF) and sweet orange (SO) leaves were collected from the greenhouse and surface disinfected.**
- **Four treatments were used to inoculate the clean leaves**
  - **Four treatments**
    - **Control (Sterile water)**
    - **Pesticide mixture**
    - **Fungal suspension**
      - **Sample OD21-42 A was used to create a fungal suspension**
    - **Pesticide mixture + fungal suspension**
      - **The fungal suspension was added 5 days after the pesticide mixture**
  - **Inoculation**
    - **Each plate had 2 leaves**
    - **The plate would be sprayed with one of the four treatments**
    - **Incubated at 28°C**
- **Only leaves treated with fungus had shown leaf damage. It is suspected the temperature difference from the incubator and the greenhouse/field could be a factor**.

# Control (Sterile water)



Sweet Orange



Grapefruit

# Pesticide Mixture

Grapefruit

Sweet Orange

# Fungus

Sweet Orange

Grapefruit

# Pesticide & Fungus

## October 15, 2021

Sweet Orange

Grapefruit

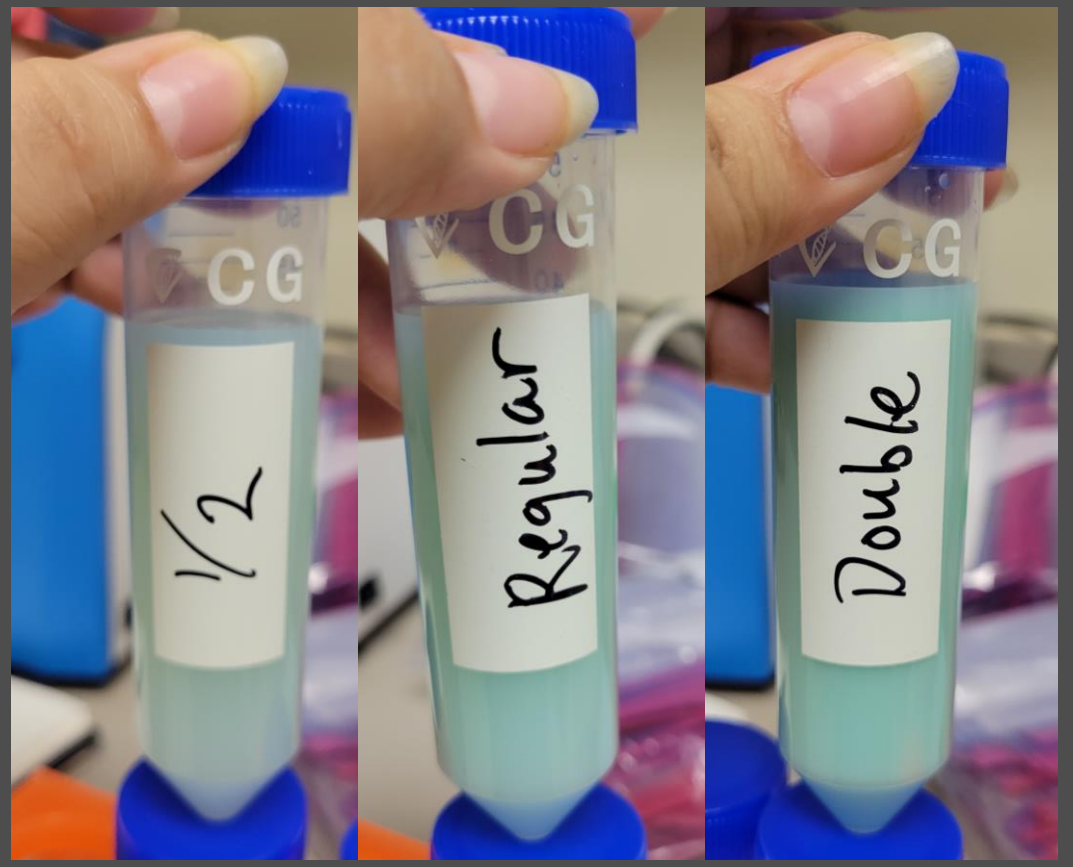
## October 21, 2021

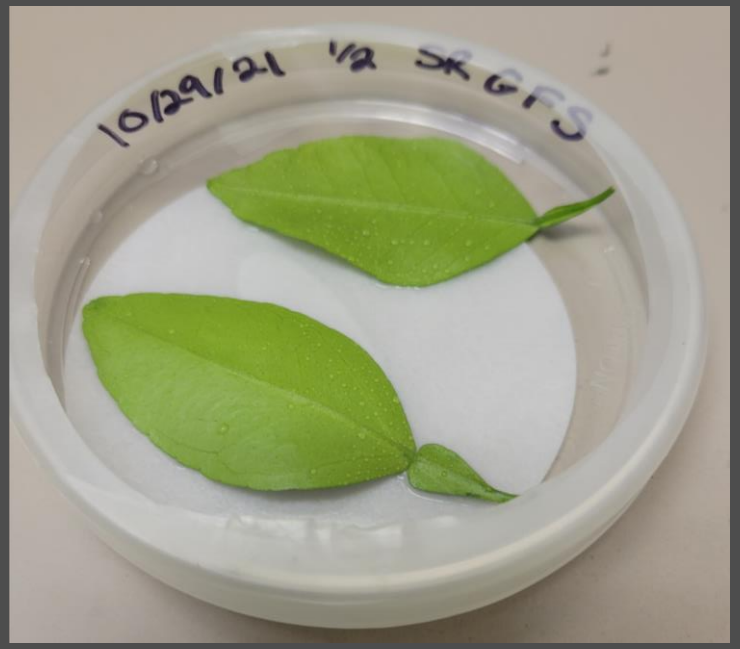
Sweet Orange

Grapefruit

# Pesticide Treatment

- **Healthy grapefruit (GF) leaves were collected from the greenhouse and surface disinfected.**
- **Three concentrations were used**
  - **Optimal Concentration (1.0x)**
  - **Half Concentration (0.5x)**
  - **Double Concentration (2.0x)**







# Pesticide Treatment

- **Inoculation**
  - **Each plate had 2 leaves**
  - **2 plates had the pesticide mixture sprayed (fine mist) on for each treatment**
    - **1 plate in incubator at 28°C**
    - **1 plate in greenhouse**
  - **2 plates had the pesticide mixture in droplets for each treatment**
    - **1 plate in incubator at 28°C**
    - **1 plate in greenhouse**

# Optimal Concentration (1.0x)

## Spray

Greenhouse

incubator

## Droplet

Greenhouse

incubator

# Half Concentration (0.5x)

## Spray

Greenhouse

incubator

## Droplet

Greenhouse

incubator

# Double Concentration (2.0x)

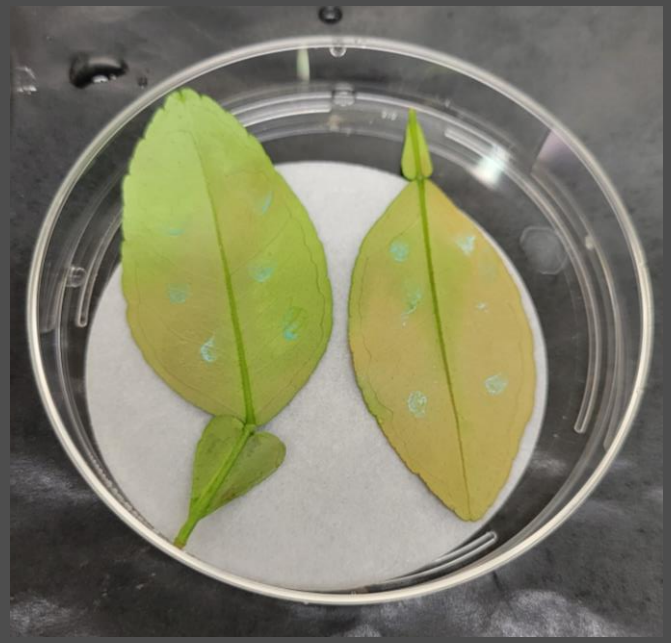
## Spray

Greenhouse

incubator

## Droplet

Greenhouse



incubator

# Discussion and Conclusion

- **Sweet Orange was shown to be more susceptible to the fungus**
- **There was fungal growth on the leaves inoculated with the fungal suspension but not any sign of damage on the ones sprayed with only the pesticide mixture.**
- **Since there were no visible damage on the leaves treated with the pesticide mixture. It was suspected that the difference of temperature from the incubator (28°C) and the greenhouse/field could be a factor.**
- **Leaves treated with the pesticide mixture in the greenhouse had shown more damage than those leaves with the same treatment inside the incubator (28°C).**

# Future work

- **Further studies will be done to characterize and identify the fungus**
  - **DNA extraction**
  - **PCR**
  - **Sequencing**
- **More studies on the phytotoxicity of the pesticides on citrus will be done.**

# Acknowledgement

**Thank you to the EFAS program, Dr. Kunta, Marissa Gonzalez, and everyone else at the Texas A&M University-Kingsville Citrus Center who helped with this project.**

# References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